PB-239 798

GUIDELINES ON SAMPLING AND STATISTICAL METHODOLOGIES FOR AMBIENT PESTICIDE MONITORING

FEDERAL WORKING GROUP ON PEST MANAGEMENT

OCTOBER 1974

DISTRIBUTED BY:

NTIS

National Technical Information Service
U. S. DEPARTMENT OF COMMERCE

BIBLIOGRAPHIC DATA SHEET	1. Report No. FWGPM-74-2	2. PB 239 798

4. Title and Subtitle
Guidelines on Sampling and Statistical Methodologies For Ambient Pesticide Monitoring

5. Report Date
October 1974

6.

7. Author(s)
Monitoring Panel

8. Performing Organization Rept. No.

9. Performing Organization Name and Address
The Federal Working Group on Pest Management
Room 431 E. Tower
401 M Street, S.W., Washington, D.C. 20460

10. Project/Task/Work Unit No.

11. Contract/Grant No.

12. Sponsoring Organization Name and Address
The Federal Working Group on Pest Management
Room 431 E. Tower
401 M Street, S.W., Washington, D.C. 20460

13. Type of Report & Period Covered
Final

14.

15. Supplementary Notes

16. Abstracts
Presents general guidelines for sampling and statistical methods for ambient pesticide monitoring. The following major chapter headings are included: Statistical and Study Design; Air; Soil; The Hydrologic Environment; Estuaries; Fresh Water Fish; Wildlife; Food and Feeds; Human Tissues. Discussions presented in each chapter include: selection of species or substrate to be sampled, criteria for sampling site selection, sampling equipment selection and care; criteria for selection of pesticides to be monitored; specific techniques; sample handling and storage. Several chapters contains literature references.

Reproduced by
NATIONAL TECHNICAL
INFORMATION SERVICE
US Department of Commerce
Springfield, VA. 22151

17. Key Words and Document Analysis. 17a. Descriptors

Pesticide Monitoring
Sampling Methodologies
Statistical Methodologies
Ambient Pesticide Monitoring

17b. Identifiers/Open-Ended Terms

Pesticide residues - air
" " - soil
" " - water
" " - estuaries
" " - fish
" " - wildlife
Pesticide residues - food & feeds
" " - human tissues

17c. COSATI Field/Group

PRICES SUBJECT TO CHANGE

18. Availability Statement	19. Security Class (This Report) UNCLASSIFIED	21. No. of Pages 51
	20. Security Class (This Page) UNCLASSIFIED	22. Price $4.25 - 2.25

FORM NTIS-35 (REV. 10-73) ENDORSED BY ANSI AND UNESCO. THIS FORM MAY BE REPRODUCED USCOMM-DC 8265-P74

FEDERAL WORKING GROUP ON PEST MANAGEMENT*

Department of Health, Education, and Welfare
J. V. Smith
J. R. Wessel

Department of the Interior
W. H. Stickel
J. E. Knoll

Department of State
C. J. Frederickson
B. L. Long

Department of Commerce
J. Lewis
T. C. Carver
F. Stein

Department of Labor
J. P. O'Neill

Department of Agriculture
F. H. Tschirley
G. G. Rohwer
K. C. Walker
R. C. Riley
J. F. Chansler

Department of Defense
H. G. Russell, Jr., *Chairman*
J. G. McWilliams
W. G. Pearson

Department of Transportation
W. F. Mitchell

Environmental Protection Agency
J. V. Osmun, *Vice Chairman*
P. H. Gray, Jr.

OBSERVERS

Council on Environmental Quality
W. Muir

Office of Management and Budget
R. Riggs

Office of Intergovernmental Relations

National Science Foundation
J. L. Brooks
E. J. Burger, Jr.

MONITORING PANEL

Department of Agriculture
H. L. Barrows, *Agricultural Research Service*
J. H. Caro, *Agricultural Research Service*
H. R. Cook, *Animal and Plant Health Inspection Service*
E. L. Cox, *Agricultural Research Service*
D. R. Embody, *Animal and Plant Health Inspection Service*
L. Heffner, *Extension Service*
F. W. Honing, *Forest Service*
A. R. Isensee, *Agricultural Research Service*
P. C. Kearney, *Agricultural Research Service*
P. F. Sand, *Animal and Plant Health Inspection Service*
M. S. Schechter, *Agricultural Research Service*
R. L. Williamson, *Animal and Plant Health Inspection Service*
J. R. Wood, *Animal and Plant Health Inspection Service*

Department of Health, Education, and Welfare
P. E. Corneliussen, *Food and Drug Administration*
J. R. Wessel, *Food and Drug Administration*

Department of the Interior
B. L. Berger, *Fish and Wildlife Service*
D. G. Donahoo, *Fish and Wildlife Service*
H. R. Feltz, *Geological Survey*
R. G. Heath, *Fish and Wildlife Service*
J. E. Seubert, *Fish and Wildlife Service*

Department of Commerce
T. C. Carver, *National Marine Fisheries Service*

Department of Defense
C. S. Bulger, *Department of the Army*
C. C. Roan, *Department of the Army*
W. W. Young, *Department of the Army*

Environmental Protection Agency
A. E. Carey, *Office of Pesticides Programs*
K. T. Mullen, *Office of Research and Development*
G. B. Wiersma, *National Environmental Research Center*
D. L. Worf, *National Environmental Research Center*

National Science Foundation
J. L. Brooks, *Division of Biological and Medical Sciences*

Tennessee Valley Authority
W. W. Barnes, *Environmental Biology Branch*
J. C. Cooney, *Environmental Biology Branch*

SECRETARIAT

W. H. Wymer, *Executive Secretary*
A. Inglis, *Assistant Executive Secretary*

* Responsible to the Council on Environmental Quality.

GUIDELINES ON SAMPLING AND STATISTICAL METHODOLOGIES FOR AMBIENT PESTICIDE MONITORING

MONITORING PANEL

FEDERAL WORKING GROUP ON PEST MANAGEMENT

TABLE OF CONTENTS

Introduction

Acknowledgement

CHAPTER

I	Statistics and Study Design
II	Air
III	Soil
IV	The Hydrologic Environment
V	Estuaries
VI	Fresh Water Fish
VII	Wildlife
VIII	Food and Feeds
IX	Human Tissues

INTRODUCTION

In the course of revising and updating various aspects of the National Pesticide Monitoring Program, the Monitoring Panel of the Federal Working Group on Pest Management identified a need for a standarized approach to sampling methodologies for the respective environmental components of the Program. Accordingly the Panel set up a task group to undertake this effort.

In preparing this report the task group imposed four restrictions on itself as it applies to the scope of the report. These restrictions were as follows:

1. The report was to be considered as a set of guidelines and not as a manual. The term "manual" implies a series of step-by-step procedures with its inferred inflexibility. The variability encountered in sampling various media from different geographical regions and with different program objectives, precludes the establishment of fixed methodologies, in a manual form.

2. The report was to apply primarily to the type of ambient or trend monitoring as defined in the charter of the Monitoring Panel. Thus, monitoring as included in the National Pesticide Monitoring Program, is defined as the repeated sampling and analysis of components of the environment to produce estimates of pesticide residue levels in these components and the changes in these levels with time

3. The report was to be designed for use by people with some familiarity with field sampling operations although not necessarily with ambient sampling for pesticide residues. Such people will most likely be associated with a federal or state agency, university or large private contractor involved with an extensive monitoring program.

4. The report was to consider both the statistical aspects of sampling as well as the field sampling procedures necessary to implement a large scale monitoring program as exemplified by the National Pesticide Monitoring Program. This Program is presently designed to include the following components: air, soil, water, estuaries, freshwater fish, birds, food and feed, and human tissues. The guidelines were to be developed around these components.

This report represents a first effort only, and the task group fully recognizes the need for updating and modifying these guidelines as time goes by. The format of the guidelines is designed to facilitate such updating.

ACKNOWLEDGMENT

The preparation of these guidelines was initiated by a task group of the Monitoring Panel, Federal Working Group on Pest Management. The task group was chaired by Dr. G. B. Wiersma, EPA, and included Dr. E. L. Cox, USDA; Mr. R. G. Heath, USDI; Mr. F. P. Kapinos, EPA; and Dr. A. R. Yobs, EPA. Additional help in the initial preparation was provided by Mr. B. L. Berger, USDI; Dr. P. A. Butler. EPA (Fla.); Ms. A. E. Carey, EPA; Mr. D. G. Donahoo, USDI; and Dr. H. Roberts, FDA. The cooperation of these persons and other representatives of the federal agencies comprising the Monitoring Panel, who made suggestions and reviewed the final draft is gratefully acknowledged. Final editing and publication of the document was done by the FWGPM Secretariat.

CHAPTER I - Statistics and Study Design

The concept of "taking a sample" is a familiar one. Loosely, it is implied that a sample is some part, generally small, of a larger entity. The concept of the sample being "representative" in the statistical sense is not so well known. It is through the quality of representativeness that information from samples can be used to make numerical statements characterizing the universe from which they come.

To conform strictly with statistical criteria, procedures for selecting a representative sample require that:

1. The total number of units or amounts of material (universe) that the sample is to represent be clearly and uniquely definable.

2. The sample or samples from the universe be selected by a method which gives all parts of the universe a definable chance of appearing (random selection).

3. More than one sample be taken from each universe under investigation unless the reliability of the sample to be representative has been pre-established.

4. If the reliability of the representativeness is not know, enough samples will be selected from the universe so that a desired level of reliability will be achieved.

It can be noted that there are problems to be solved by sampling which do not require that the samples be representative. Frequently in regulatory work the aim will be to determine the maximum (or possibly minimum) value which can be found in a sampling unit from a given universe. This aim can be met at minimum cost by following principles of random selection and designed search.

A sampling program may be properly designed to provide adequate definition of local conditions or universes. It is generally not possible to consider these local universes as subuniverses of a broader or national universe. Many mistakes have been made by not recognizing this fallacy. If, however, the large universe is considered in the design and the subuniverses are selected by statistical principles then representative samples from these subuniverses can be used in making inferences about the large universe.

A basic premise in statistical thought is that many phenomena are incompletely or inadequately described by a single measure. The average, mean, median or some other measure of centrality is too often used as an index for description of a set of samples where much of the information in the set reflects the spread around the central value. Statistical usage insists that estimates of at least two parameters be considered for descriptions based on sample information. The two parameters most frequently estimated are know as mean and standard deviation.

Underlying the specification of estimates of two parameters for definitions is the concept of distribution or probability distribution. Even a well defined universe from which a large number of samples can be taken would exhibit for all possible samples an almost continuous spectum of sample values against probability of attainment of these values. Hence, if enough random samples are taken from a universe, a good definition of the possible values from that universe can be made as well as estimates of the chance that certain specified values will be exceeded.

For example, suppose that the residue level of a pesticide in the eggs produced for human consumption in a state during a given period can be considered as a well defined universe. A well designed program of taking random samples from this universe would provide an approximation of the distribution of these residue levels and from this approximation, the chance that residues exist in excess of any prescribed level can be estimated with an order of reliability dependent on the number of samples used in defining the distribution.

The challenge might be raised that the method described above presents the probability that levels of residues exist which have not been observed in any sample. This is true. For example, a set of samples might contain, no observed residues above 0.1 ppm but the estimation of a distribution to fit the set of samples might imply 1% of all possible samples could be in excess of 0.1 ppm. The examination of the reliability of this estimate might result in the confidence statement "there is a 5% chance that more than 1.3% of all possible samples could have values in excess of 0.1 ppm". If the implications of this result are serious, the estimates and the confidence statement can be improved only by obtaining additional and properly designed sampling information.

The general principles implied here will be basic for obtaining and treating sample information from all components of the natural environment. Certain components will introduce special problems and require special methodology to meet the general principles.

The need for a universe to be well defined has been referred to above. It is easily recognized that the more homogenous a universe is, the number of samples needed to provide an adequate description of that universe will be smaller. Thus, it is generally advantageous to divide a universe, prior to samplings into subunits which are known to be more homogenous within themselves, when this can effectively be done. This valuable concept is called stratification.

As an example, in the national plan for sampling pesticide residues in soils, two strata were defined. These strata were called cropland and noncropland. The names are somewhat self explanatory and the fact that somewhat different pictures are expected of the pesticide residues in the two strata is readily recognized. At the time the project was planned,

subdivisions into many more strata were proposed. These were difficult to define clearly and hence were not considered. The principle was correct but the means for achievement were unavailable.

In planning an investigation to define the levels of some deleterious element in a component of the natural environment on a regional or national basis, it would be advisable to review all the recorded data now in existence. It will generally be found that these data have not come from a program designed according to statistical principles. Hence it will be incorrect to extrapolate from these results knowing that if such an action is tried, no assessment of reliability can be given for the estimates presented. Nevertheless, these data need to be examined to get some feeling as to the type of values that may arise. Also the records will give valuable information for defining strata and estimating numbers of samples needed for a planned investigation. When all available background is considered, a pilot study for some region or stratum should be planned. While the pilot study is being expedited, the planning for an operational program can begin. The latter may need to be modified after the results of the pilot study are assessed. A program for a national study needs frequent points at which the results will be reviewed, and an understanding that modifications will be quickly made when these results suggest that preconceptions may not have been correct. There is a concept very widely held that after a plan for an investigation is presented it should never be altered no matter how badly it works. Many programs have produced less than optimum results through this wrong-headedness. It is just as easy to produce and operate a program which permits feedback and modification when the nature of the findings indicate that changes are desirable.

A caution must be kept in mind in dealing with the type of data that arises from most investigations of residues. Any well designed program is likely to show most of the sample results to be in the "nondetected" category. This means that generally a rather large number of samples must be taken to achieve a reasonable picture of the distribution. A well defined estimate of the mean or other central value is generally difficult to express. The types of summary statements depending on assumptions of the distributional form have not received full acceptance. Unless an acceptable alternative can be proposed, the methods based on a distributional assumption is recommended.

In applying the above principles to the evaluation of pesticide residues in soils by a sampling program, we immediately recognize a potential difference in distribution depending on whether persistent or nonpersistent chemicals are under consideration. The least elaborate program can be designed for the definition of the persistent chemicals. Stratification according to two or more use levels would be in order.

The problem of detecting changes with time can be approached by taking samples at the same locations at intervals of several years if no

additional pesticide is applied in the interval. If the application is yearly, the sampling program needs to be modified accordingly.

In dealing with nonpersistent chemicals, the time since application needs to be considered in providing an appropriate stratification system. Changes with time in terms of years may have little interest but changes by month or even days may be the information desired.

Even if yearly or more frequent applications of a chemical under consideration is the nature of its use, the date of application is an element to be considered in making a meaningful plan for its evaluation. A general plan to provide estimates for both the easy and difficult problems needs to be primarily guided by planning to meet the difficult.

The general evaluation of pesticide residues in water is inherently more difficult than for soils. Thus separation into watershed units neglects consideration of samples from large rivers. A succession of samples along the course of a large river might suggest successive integrations which would permit earlier fractions to be subtracted but the validity of this procedure needs to be established. It has been pointed out that the sampling of waters may be considered not only for estimating the residue load coming from the land but also for estimating the residues headed for lakes or the sea. For many residues, however, a lengthy time of transport may change their character by deposit, chemical reaction, etc., and hence a good definition of upstream waters may not provide a good predictor of the quality further downstream. Experience in the Hudson and Chesapeake estuaries has demonstrated that an integrated sample across the estuary at any time would be a poor indicator of the nature of the waters at some other time. An integrated sample cross channel and over a full tidal cycle is needed for a firm measurement.

The basic geographic stratification for an air sampling program should recognize regions with larger concentrations of populations and/or industry as needing the heaviest sampling rate. These regions should be mapped as larger than standard metropolitan areas. Possibly two additional strata; low and moderate population densities should be considered.

The problem of recognizing classes of prevailing meteorological conditions needs to be met. Certain areas such as southern California and the Great Basin are examples of air masses that have a tendency to be stationary for lengthy periods and which form a recognizable category. There are other areas where such stagnation may exist for only short periods. How these conditions should be considered for planning purposes is uncertain.

The foregoing discussion outlines the range of statistical considerations which a planner must draw upon in setting up any sampling procedures for a monitoring program. Specific choice of statistical methodology and computational details for such methodology are obtainable from several basic texts. A brief list of the more widely used texts is provided.

REFERENCES

Statistics and Study Design

Cochran, W. G. Sampling Techniques, 2nd ed. Wiley, Ney York (1963).

Hansen, M.H., W.N. Hurwitz, and W.G. Madow. Sample Survey Methods and Theory. Wiley, New York (1953)

Kish, L. Survey Sampling. Wiley, New York (1965).

Sampford, M.R. An Introduction to Sampling Theory. Oliver and Boyd, Edinburgh (1962).

Snedecor, G.W. and W.G. Cochran. Statistical Methods, 6th ed. The Iowa State University Press, Ames, Iowa (1967)

Yates, F. Sampling Methods for Census and Surveys, 3rd ed. Charles Griffin, London (1960).

CHAPTER II - AIR

Monitoring ambient air for pesticide residues is technically difficult at present because the fundamental technology necessary for such a program is still being developed. The physical-chemical behavior of airborne residues, i.e., duration of vapor stage, factors governing particulate adsorption/absorption and desorption, influence of water vaper, etc. and the pattern of geo-physical effects are all largely unknown and must be defined to optimize sampling efficiency. Ambient air monitoring is also difficult because it is possible that an unlimited range of pesticide residues are present, requiring identification and measurements at microquantity concentrations that vary widely with geography, season, local pesticide usage and altitude. Various nonpesticide air pollutants may also be collected during the sampling process and may subsequently interfere with analysis.

Specific guidelines for air sampling in connection with monitoring pesticide residues in ambient air cover four areas listed below.

PROGRAM DESIGN

Goal Identification: Clear definition of the purpose of the contemplated monitoring program is essential to the success of the program and will direct many of the decisions required in preliminary planning.

Sampling Site Criteria: The criteria for sites will be guided by and are important to attaining the program goal. Criteria must be selected after considering a variety of conditions such as geographic divisions, prevailing weather patterns, pesticide usage patterns, cropping population concentrations, elevation above ground level or above sea level, etc. Service requirements such as power supply and travel time must also be considered. The attributes of sampling sites must be defined before a monitoring design is developed.

Statistical Design: This is most important in assuring that collected data is unbiased and can be treated statistically to determine the significance of findings. The statistical design provides for the selection of sampling sites on a random basis, insuring that equal consideration is given to sites that meet the established criteria.

Nature of Sample: A sample of air may be collected continuously or intermittently depending on restrictions imposed by monitoring goal, program design, sampling equipment capability and analytical factors. The parameters of a sample must therefore be defined in these terms and expressed as a volume of air to be sampled.

Sampling Frequency: Samples may be collected as often as indicated by the established monitoring goal and statistical design so long as this is consistent with available laboratory support.

Ancillary Information: Measurements of such variables as temperature, windspeed and direction, precipitation, etc. along with information about the sampling locality and pesticide usage is helpful in the interpretation of ambient air monitoring results. Need for or value of such ancillary information must be predetermined and arrangements made to obtain this information at the time of sampling.

ANALYTICAL SUPPORT

Sampling and analysis are distinct parts of any monitoring program. They are interdependent, however, since each controls certain aspects of the other. It is necessary to make certain decisions about the analytical phase at this point in program planning because these decisions will control such things as the volume of air to be collected in each sample, the maximum tolerated delay between sample collection and analysis, and sample handling, including transport and storage.

Residues to be Monitored: The variety of pesticides potentially present in ambient air together with the limited number of multi-residue techniques available means that target residues must be carefully selected to meet program needs within available analytical support limitations. This also is important in selection analytical procedures to be used.

Analytical Procedures: These must be of such sensitivity and specificity to permit detection and identification of the desired compounds at the levels at which it is anticipated that they will be present.

Confirmatory Procedures: These are necessary because of the unlimited variety of pesticide residues potentially present. Such procedures are generally less sensitive than the basic analytical procedures and require additional analytical time. The investment of these resources is imperative, however, to substantiate the identification of the residues.

Reporting: Details must be established for reporting analytical findings, including units of reporting (weight per volume of air, particulates, etc.). These should be selected to assure reporting in whole numbers as much as possible.

SAMPLING EQUIPMENT

Sampling Equipment must be selected which can operate within the criteria established for the site.

Construction: Equipment should be constructed for durability in a variety of climatic conditions, yet be light enough to facilitate transportation. It may be constructed of any materials as long as the air stream is not exposed to contaminants or otherwise modified prior to sampling.

Calibration: Each sampler should be tested upon installation in the field to assure that it is working properly and to calibrate the actual air flow air volume sampled.

Pre-testing: Equipment and trapping reagents should be tested with regard to the selected residues for sampling efficiency, and retention at the planned air flow rate.

FIELD OPERATION

Action must be taken to assure field operation by the desired parameters.

Sampler Location: This should be standardized. The intake passage should be as short as possible to decrease residue loss through absorption at the inlet. There should be free access on all sides to ambient air. This should be no less than 5 feet above ground level to avoid surface turbulence. If external power is required, a source must be available.

Servicing: A schedule should be established for regular servicing and preventive maintenance as indicated by the sampling equipment and schedule. Glassware should be cleaned after each sampling interval.

Sample Handling, Storage and Transport: Do not disregard the importance of these in the overall program. They are vital in preserving collected residues for analysis. Collecting reagents should be placed in chemically clean screw top glass containers with aluminum foil or other non-reactive liners and refrigerated. Transport to the laboratory should be by the most rapid practical method.

CHAPTER III - Soil

It is the purpose of this chapter to review techniques of soil sampling capable of application to pesticide residue estimations in soil: the types of sampling equipment, shipping and storage, and soil properties used for correlation with residue levels. Most of the information is drawn from the open literature, as well as from the methodologies of government agencies actively involved in monitoring pesticide residues in soil.

Compared with analysis for soil nutrients, sampling for pesticide residues is relatively new. However, many of the basic concepts which guide sampling for nutrient studies can be utilized in residue monitoring. Cline (1) in his classic work on soil sampling, endorsed the concept that soil should be considered a volume to be sampled, not merely an area. Soil is also a heterogenous medium, i.e., most of the physical and chemical properties are not uniform along all dimensions. However, within a limited spatial area, a soil body can also be considered homogeneous, with respect to soil type, vegetation and cultural practices. Therefore, any good sampling plan must recognize and attempt to minimize these variations in order to obtain a sample which can give an accurate estimate of the parameters under investigation.

GENERAL SOIL SAMPLING CONSIDERATIONS

Sampling Error vs. Analytical Error

Several studies have demonstrated that field error is much greater than analytical error in soils testing (1,2,3,4). Therefore, the field sampling methodology should aim at minimizing this error.

Disturbed vs. Undisturbed Profiles

On cultivated agricultural land, cultivation has mixed the upper horizons of the soil to a fairly homogeneous layer called the plow layer or furrow slice. Organic matter, usually concentrated at the surface is uniformly incorporated. On uncultivated land or forested land, the layer of organic matter is usually present at the surface and acts as an efficient receptor and retainer of many pesticidal compounds. Organic matter is a highly ionic soil fraction and, along with the clay fraction, is responsible for most of the adsorptive capacity of a soil. A large percentage of the pesticides applied to this type of site will be held in the organic layer. Depending on the main objective of sampling, uncultivated lands may be sampled by one of the following methods:

a. Collecting separate samples of both the organic layer and the mineral soil horizon beneath it.

b. Collecting a composite sample in which the organic and mineral soil fractions are mixed and treated as the sample.

c. Removing the organic layer at each core site and sampling only the mineral soil.

Urban Soils

Urban areas present unique problems for soil sampling. Here distinct profiles may be almost non-existent. Practically nothing would be gained by sampling deeper than the top 5-8 cm (2-4 inch). Sampling personnel should be aware that the surface layer of urban soils often contains a number of foreign contaminants, such as broken glass, nails, bricks, etc., which should be eliminated by sieving. For samples contaminated with oil or other non-natural liquids, notation should be made on the sample collection forms so that analytical procedures can be appropriately modified.

Depth of Sampling

For most pesticide sampling situations, collecting soil to a depth of 5-8 cm (2-4 inches) is sufficient. Most of the persistent pesticides and many of the less persistent pesticides remain at or near the surface.

Sampling Horizons vs. Set Depths

If sampling at depths greater than 30 cm (12 inches) must be done (as in monitoring for a water-soluble compound), it is advisable to collect samples at pre-selected depths from the surface rather than attempt to sample a particular soil horizon(s). Most field personnel do not have the necessary expertise in soils to identify particular soil horizons over a variety of soil types and geographical area. In addition, the costs for this type of sampling method would be prohibitive for all but the most limited applications, since a trench must be dug to expose the profile or specialized equipment such as a split-tube sampler or earth-drill-bucket auger combination must be used.

SPECIFIC CONSIDERATIONS FOR PESTICIDE RESIDUES

Contamination - In any sampling program for pesticide residues. Contamination of samples presents a significant source of error. Since soil is an aqueous system and most pesticides are either insoluble or only slightly soluble in water, the probability of carrying residues from one sample site to another on sampling equipment, vehicles and clothing is high. The use of the following proper sanitation procedures cannot be overemphasized:

> All tools and equipment used in residue monitoring should be constructed of metal and be easily cleanable. Wood should not be used for constructing equipment. Tools must be thoroughly cleaned immediately after sampling a site. Scrub with a brush and water, then rinse with an organic solvent such as alcohol or acetone.

Sampling tools and equipment ideally should be wrapped in clean cloth bags and subsequently transported or stored in large, heavy-gauge plastic bags, sealed with masking tape or cord. They must not be stored near any pesticides or in a building used for storing pesticides.

Vehicles which have been used to haul pesticides should be avoided for collecting samples if at all possible. Before using any vehicle, it should be thoroughly cleaned inside and out with soap or detergent and hot water and rinsed.

Clothing, gloves and shoes that have been exposed to pesticides should not be worn when sampling unless they have been thoroughly cleaned. Neoprene work gloves can be used in sieving soil but must be washed with alcohol and air dried between samples.

Type of Pesticides - The properties of the particular pesticide or group of pesticides under investigation will determine many aspects of the sampling program and the subsequent handling of the samples. The following should be considered:

Persistent - Samples containing less persistent compounds may have to be collected sooner, given special handling and extracted sooner than samples containing persistent compounds.

Solubility, Volatility, etc. - Water-soluble compounds are likely to be found slightly below the surface rather than right at the surface.

Use - Placement of pesticide in relation to target species or crop affects the spatial distribution of the soil sample.

GENERALIZED SAMPLING METHOD

Statistical Design - Unless a sampling program has a statistical basis, the data generated by it is not likely to be representative of the area sampled and consequently the effort will be wasted. The most advanced analytical methodologies cannot improve upon a poorly taken sample. In general, the sampling sites should represent a percentage of the total areas to be sampled (i.e., one sampling site per specified area of land). Total area sampling is essentially impossible as well as wasteful of both time and money, and the increase in precision cannot justify the resource expenditures.

Site Size and Number of Cores - In general, the number of cores should be enough to adequately sample the variation present within the site. The National Soils Monitoring Program, operated by the Envrionmental Protection Agency, uses the following site sizes and core numbers:

	SIZE	NO. OF CORES
National program (cropland)	4 ha (10 acres)	50
Urban monitoring	225 sq. m (270 sq yds)	16

Core dimensions: 5 cm (2") in diameter x 8 cm (3.5") in depth.

The cores should be taken in a grid pattern over the entire site. The grid insures uniform coverage of the site and is easier for nontechnically-trained, field personnel to master.

Compositing - For assessing the residue levels on several sites over a large geographic area, compositing the individual cores from each site to one sample is recommended. It is the most economical method of obtaining the mean residue value. If any statistic other than the mean is desired, the composite sample should not be used.

Sample Preparation - The soil cores taken from the site should be sieved and thoroughly mixed. Although a 10-mesh sieve (2 mm opening) would be preferable, a larger mesh should probably be used to compensate for the wide range of moisture conditions encountered in the field (the National Soils Monitoring Program uses a 3-mesh sieve with 6.3 mm opening). If the sample is too wet to sieve, it should be air dried on aluminum foil until sieving can be accomplished. Sieving removes the coarse soil fractions and undecomposed organic debris. The sieved sample should be sub-sampled as necessary to fill the sample container, sealed and labeled. All sample containers should be clearly labeled as to site location, material class and date. Site locations should be marked on a county map or otherwise recorded. If agricultural land is being sampled, a pesticide use history should be obtained from the landowner or operator. This use history should be sent with the sample to the analytical laboratory.

SAMPLE SHIPPING AND STORAGE

Proper handling and storage of samples after collection is just as important as proper sampling techniques. Careless shipping or storage can lead to sample contamination or degradation of the sample.

Sample Containers - Containers for pesticide monitoring samples should protect the integrity of the sample during transport. There are several kinds available.

Metal - Metal containers offer a rigid structure that can protect the sample during shipping. They should be rinsed in acetone or alcohol and air-dried before using. Metal containers may be more expensive.

Plastic or Polyethylene - Plastic or polyethylene bags are light, easily carried and are usually lower in cost than metal containers. They do not offer the sample protection that metal containers do and therefore, the samples must be more carefully packed for shipment. Plastic bags should be checked for PCB content. Certain polyethylene containers are also capable of absorbing some pesticidal compounds. This should be checked before using.

Sample containers should be transported from site to site in plastic bags, or otherwise protected from airborne contamination.

Sample Transport and Storage - The subsequent shipment and storage of samples is partly dependent on the particular pesticides under investigation.

Freezing - Freezing is probably the best method of sample preservation for most pesticides. Samples should be frozen with dry ice in a large picnic chest as they are collected. They can be shipped frozen and covered with dry ice in styrofoam boxes. Once at the laboratory the samples should be held in large freezers for extraction and analysis.

Shelf-storage - Many persistent pesticides are not greatly affected by room-temperature storage. Such samples can be held 1-2 years on shelves at approximately 20-23 degree C with little degradation. Samples containing nonpersistent pesticides should either be stored in a freezer or extracted and the extract frozen.

SAMPLING EQUIPMENT

Equipment for sampling is varied. The sampler chosen should provide a uniform, reproduceable unit. The most commonly used types in soil sampling are blades, augers and tubes.

Blades - Blades or shovels are not recommended for use in a pesticide monitoring program. Uniform volumetric units for a composite sample cannot be collected with this type of sampler.

Augers - Bucket augers may be used, particularly when samples are collected at depths greater than 30 cm. However, there is the potential of contamination of lower layers with material from upper horizons. Soil at the top and bottom of the bucket should be removed and discarded to minimize this problem.

Tubes - The tube (or core) sampler is probably the most commonly used sampling device. Sampling tubes provide a core with a uniform cross section, minimize contamination and permit rapid sampling of most soils. They can be difficult to use in stony, dry, very heavy or very

sandy soils. Most tube samples tend to be slightly compacted. Open-sided tubes permit observation of the core before removal. The most often used diameters are from 2.5 - 7.6 cm (1-3 inches). A uniform depth of sampling can be maintained by painting a line on the side of the tube at the desired depth or fabricating a metal collar to restrict vertical penetration.

A few types of split-tube samplers can also be either purchased or fabricated. These consist essentially of a tube within a tube. Most are used in connection with a power auger system. After the core is taken, the outer tube is removed and the actual sample used is that contained within the inner tube. This method is very good for minimizing contamination between soil horizons, but is also much more expensive, and time-consuming. Its use for most pesticide monitoring situations is not recommended.

SOIL TESTING

The fate of pesticides in the soil is determined by several factors, including the physical and chemical properties of the medium itself. Most adsorption of pesticides in soil can be correlated to the percent organic matter and the clay content. A few simple tests can give a more complete picture of the soil-pesticide complex. The properties most often tested are: percent organic matter, soil texture and pH. Methodologies for all tests are given in many handbooks (5,6)

Percent organic matter - Loss on ignition is the simplest method and requires no special equipment or chemicals. Each sample is weighed, the organic carbon is burned in a muffle furnace, and the sample is weighed again. If large numbers of samples are to be tested, the chromate titration method (Walkley-Black) may be used.

pH - Values for pH can be determined using a pH meter. The determination is relatively quick and simple; it is easily adapted to large numbers of samples.

Soil texture - Texture of soils is expressed in percentages of sand, silt and clay. The hydrometer method is generally the easiest to use.

LITERATURE CITED

1. CLINE, M. G. 1944. Principles of Soil sampling. Soil Sci. 58:275-278

2. REED, J. and J. A. RIGNEY 1947. Soil sampling from fields of uniform and non-uniform appearance and soil types. J. Amer. Soc. Agron. 39:26-39.

3. CLINE, M. G. 1945. Methods of collecting and preparing soil samples. Soil Sci. 59:3-5

4. RIGNEY, J. A. and J. REED 1946. Some factors affecting the accuracy of soil sampling. Soil Sci. Soc. Amer. Proc. 10:257-259.

5. BLACK, C. A. ed. 1965. Methods of Soil Analysis. Part I: Physical and Mineralogical Properties, Including Statistics of Measurement and Sampling. Part 2. Chemical and Microbiological Properties. Agronomy Monograph No. 9 American Society of Agronomy, Madison, Wisconsin.

6. WILDE, S. A., G. K. VOIGT and J. G. IYER 1964. Soil and Plant Analysis for Tree Culture. 3rd revised edition Oxford Publishing House, Calcutta, India. 209 pages.

CHAPTER IV - THE HYDROLOGIC ENVIRONMENT

Pesticides are distributed throughout the hydrologic environment--the atmosphere, precipitation, surface water, ground water, suspended and bed sediments, flora, and fauna. Measurements in each component are equally important, and validity begins immediately upon sampling. Irrespective of scrutiny and quality control applied in performing laboratory analyses, reported data are no better than the confidence that can be placed in the representativeness of the sampling (1).

This chapter directs attention to the occurrence of pesticide residues in water and sediments, sampling techniques for obtaining representative samples, and sample handling and preservation.

OCCURRENCE OF PESTICIDE RESIDUES

Pesticide residues have been detected in all components of the dynamic hydrosystem, attesting to the ubiquitous nature of the residues. The occurrence and distribution of residues are as divergent as the components. Concentrations range from the generally accepted lower detection limit of 0.01/ug/l (micrograms per liter) in filtrates to 100,000 times this value in particulate matter separated from the same aqueous samples. The environment ranges from the most humid to the driest. Residues may originate from local applications, industrial or urban discharge, or as fallout transported from remote areas.

Preliminary results of an investigation of pesticide residues in runoff to streams draining different land-use areas in central Pennsylvania indicated that the concentration of the DDT family sometimes is directly correlated with suspended-sediment concentration(1). This is particularly true for the chlorinated hydrocarbon pesticides which are only sparingly soluble in water and tend to sorb on sand and clay particles being transported and/or deposited in the water body.

In the case of the more soluble pesticides, including organophosphate and carbamate insecticides and many of the herbicides, dispersion of the pesticide residues is more dependent on the vertical and lateral mixing within any given cross section of a body of water. The hydrologist collecting the samples must, therefore, not only be familiar with the mixing characteristics of streams and lakes, but also have a good understanding of the role of fluvial-sediment transport and deposition.

SITE SELECTION

Many factors are involved in the proper selection of sampling sites, including the following: objectives of the study; accessibility; flow, mixing, and other physical characteristics of the water body; pesticide source locations; and personnel and facilities available to conduct the study.

Objectives - Different agencies will have different objectives for determining the concentrations of pesticide residues in streams or lakes. Assuming that most water-oriented studies are based on evaluating the effects of overland runoff, ground water inflow and/or waste disposal into our waterways, the study can usually be tied to the physiographic features of the area under consideration. In most cases, the quality of water flowing into a lake or past a particular point along a stream can be related directly to inputs within the drainage basin which includes the entire drainage area upstream of a selected point. Therefore, whether the basic objective for the monitoring program be reconnaisance, long-term trend evaluation, or solving specific problems, the first step in the study is to define the appropriate hydrological boundaries and then establish the sampling site locations above and/or below these boundaries accordingly.

Accessibility - Accessibility to any sampling site is an obvious requirement and can often be directly related to sampling costs. Bridges are normally the first choice for locating a sampling station since they not only provide ready access to a stream but they also permit sampling at any point across the width of the stream. Sampling locations on lakes as well as those on larger rivers may require the use of a boat. Frequently, however, the use of a boat will not only take more time in traversing a stream, it may prove difficult in the manipulation of the sampling equipment.

Physical Characteristics - The ideal sampling station would be a cross section of a stream (or lake) at which samples from all points on the cross section would yield the same concentrations of all constituents, and a sample taken at any time would yield the same concentrations as one taken at any other time (2). This situation never persists in nature for any length of time which points to the need for careful site selection which will, as nearly as possible, provide uniform flow and good mixing conditions. Any uncertainty regarding the uniformity of flow or the completeness of lateral and vertical mixing may be resolved by taking multipoint, depth-integrated samples and compositing the samples prior to analysis.

Availability of streamflow and/or sediment discharge records can be an important consideration in the selection of pesticide sampling sites on streams. Adequate discharge data are essential for estimating the total loads carried by the stream. If a gaging station is not conveniently located on a selected stream, the hydrologist should explore the possibility of obtaining discharge data by direct or indirect means.

Source locations - The hydrologist must be aware of the locations of any point sources of pesticide discharges (such as formulating or manufacturing plants, sewage outfalls, etc.) so that he can select sampling sites which would reflect the conditions set forth in the

objectives. Representative measurements of a pesticide load at a point on the main stream that is close below a source of waste inflow or a tributary stream is highly impractical. The inflow frequently hugs the stream bank with very little lateral mixing for some distance. A solution for avoiding this situation is to select a station site above the waste source or tributary stream, or to move the site far enough downstream to allow for adequate mixing.

Personnel and facilities - The sample collector visits the stream or lake under investigation more than anyone else involved in the study. An experienced, observant, and conscientious fieldman can contribute a great deal to any monitoring study. Therefore, anyone who wishes to achieve good proficiency in the planning and conduct of monitoring programs will be well-advised to spend a reasonable apprenticeship in the job of sample collection.

The fieldman should make all preparations possible before starting on his sampling assignment. This is best accomplished from a centrally located field office where a workshop is available to make any necessary repairs or adjustments to sampling equipment, boats, outboard motors, trucks or cars as appropriate.

SAMPLING TECHNIQUES

Water - Sample collection in the water column varies in degree from the simplest of hand sampling procedures at a single point to the more sophisticated multipoint sampling techniques known as the equal-discharge-increment (EDI) method or the equal-transit-rate (ETR) method (3).

Generally, the number and type of samples to be taken depends on the width, depth, stream discharge, and the suspended sediment being transported. In the sampling of lakes, a number of samples can be collected at selected grid points and then composited or a single sample can be collected near the center of the water mass, depending on the size of the lake. In any case, the larger the number of individual points sampled the more nearly will the composite sample represent the water body.

Water is often sampled by filling a container held just beneath the surface of a body of water. Published data and discussions by investigators reveal that a high percentage of samples have been obtained in this manner (commonly referred to as a dip or grab sample). Using a weighted-bottle holder which allows the bottle to be lowered to any desired depth and returned to the surface improves on this method. If the bottle is lowered to the bottom and raised to the surface at a uniform rate, the resulting sample will roughly approach the collection of what is known as a depth-integrated sample.

A true depth-integrated sample is collected by means of a depth-integrating sampler which integrates discharge as a function of

depth. Sediment is maintained in suspension because of velocity and turbulence. Velocity varies from the water surface to the stream bed, being generally highest near the surface and lowest at the bed. Sediment concentration varies also from the surface to the bed, being lower at the surface and greatest at the bed. Fine sediment (finer than about 0.062 mm) is easily kept in suspension and is distributed relatively uniformly throughout the depth of flow. However, as particle size increases, more energy is required to maintain suspension in a given flow, and the average size of sediment in suspension increases from the surface to the bed. Depth integration is used to collect a water-sediment sample that is weighted according to velocity at each increment of depth. This means that the water-sediment mixture must enter the sample container at the same velocity as the flow passing the intake. If the depth-integrating sampler is lowered from the surface to the bed and back at the same transit rate, each increment of flow in that vertical is sampled proportionately to the velocity.

The open-mouth weighted bottle sampler, therefore, does not collect a truly representative sample in a flowing stream if there are many particles coarser than about 0.062 mm carried in suspension. Another disadvantage in using an open-mouth bottle sampler in flowing streams is that there is no assurance as to when the bottle becomes filled, compounding the uncertainty that the collected sample truly represents the distribution of both dissolved and suspended material in the sampled water column. This method of sampling may be extremely poor for flowing streams but may be used effectively for slow-moving bodies of water such as ponds, lakes, and estuaries.

One sampling technique currently accepted by hydrologists for use in sand-bed streams is the equal-transit-rate (ETR). With this method standard suspended-sediment samplers are used to collect a velocity-weighted sample. Samples are taken at a number of equally spaced verticals in the cross section depending on stream width. The transit rate of the sampler, which is the rate of movement of the sampler from the surface to the bed and back to the surface, is the same at all verticals. Samples collected in each vertical are all composited into a single sample representative of the entire flow in the cross section. In this manner, the composite sample of the water-sediment mixture flowing in the cross section is velocity- and discharge-weighted.

It is recommended that all water samples be collected using depth-integrating methods whether the samplers are hand held or cable-and-reel suspended. The only exception would be in the case of very shallow streams where the depth is insufficient to allow true depth-integration. In such cases dip samples collected at one or more verticals across the stream would be appropriate; however, the sample container should be carefully held beneath the water surface in order to avoid stirring up and entraining bottom sediments.

The number of verticals at each sampling site will usually be decided by the fieldman. For small streams, a depth-integrated sample taken at a single transverse position located at the centroid of flow is usually adequate. Larger streams require selection of several verticals at centroids of equal-discharge-increment (EDI). This method requires that the fieldman have some knowledge of the streamflow distribution in the cross section before sampling verticals can be selected. To make the EDI measurement when prior streamflow data is not available, it is first necessary to determine the total discharge across the stream channel and then subdivide the cross section according to incremental discharges.

The number of verticals required for the equal-transit-rate (ETR) method depends on the streamflow and sediment characteristics at the time of sampling as well as on the desired accuracy of the result. For all but the very wide and shallow streams, twenty verticals are usually sufficient.

Obviously, the multipoint sampling techniques can become very time consuming and, consequently, expensive. An alternate method often used consists of sampling at the quarter points or other equal intervals across the width of the stream. Composite samples obtained from individual samples collected at the quarter points can be fairly representative in most cases, providing that the stream cross section was properly located.

Lakes can be sampled on three-dimensional grids consistent with the shape and depth of the water body; however, small ponds or lakes are usually sampled at a single vertical which should be near the center of the water mass. Distribution of sampling locations in large bodies of water will be largely dependent on manpower and financial restraints.

Bottom Materials -- Bottom sediment samples are usually taken at the same horizontal locations at which water samples are collected. A bottom sediment sample may consist of a single scoop or core, or be a composite of several individual samples in the cross section.

Pesticides sorbed in bottom material, irrespective of the ratio of inorganic to organic composition, may reflect an integration of chemical and biological processes. They serve the indispensable historical role in reflecting input to non-scouring streams, lakes, and estuaries with respect to time, application of pesticides, and land use. Recalling that fluvial materials tend to settle out during periods of low streamflow or of calm conditions in lakes, and are additive to solids that have accumulated on stream beds or in lake bottoms, periodic sampling of water overlying these deposits might not reveal the presence of pesticides, yet they may be found in the solid material which acts as a sink and reservoir.

The loss of low-density deposits must be kept minimal during any sampling process, requiring a bottom-material sampler that is capable of collecting and retaining the "fines" which sometimes contain the highest concentration of pesticide residues.

Few data have appeared in the literature regarding the presence of pesticide residues at the liquid-solid interface or in the hydrosol area. Depending upon ambient physical, biological, and chemical controls, residues may be transformed into metabolites, degraded, or taken into solution. Further study of these processes is an open and challenging field of endeavor. An evaluation of this portion of the hydrosystem is essential to comprehensive assessment of the occurrence and distribution of pesticides.

Sampling Devices - To overcome the problem associated with collecting representative samples, equipment that has been specifically designed and thoroughly tested is favored. In most instances, samples for pesticide residue analysis are best collected with the same equipment and techniques used for the collection of suspended-sediment samples. Several depth-integrating samplers are available and suitable for this purpose. In shallow streams and wetlands that can be waded, the US DH-48 suspended-sediment sampler can be used successfully. The US DH-59 suspended-sediment sampler was designed to be suspended by a hand-held rope in streams too deep to be waded. Both of these samplers are simple and of clean design using a container which is easily inserted into position and held firmly by spring action. The US D-49 suspended-sediment sampler has been used for many years in collecting depth-integrated suspended-sediment samples in the larger streams and rivers. It is provided with a choice of nozzles - 1/8, 3/16, and 1/4-inch in diameter, with which inflow of the water-sediment mixture can be controlled. The D-49 sampler, which weighs about 60 pounds, is suspended on a cable and operated with a reel attached to a boom.

The open-mouth weighted-bottle sampler, which is available in many sizes, is acceptable for use in lakes and slow-moving streams having little suspended sediment. The sample bottle is inserted into a weighted holder to which a rope is attached as a means for suspending the sampler.

Bottom deposits present a more difficult set of sampling conditions than those in the water column in streams or lakes, primarily because of the varying firmness of bed materials. The US BMH-60 bed-material sampler is a 30-pound impact type sampler designed primarily for use in sand-bed streams. However, it works equally well for firm and partially consolidated bottom materials. One man can collect samples with this sampler under most conditions. The bucket takes a bed-material sample that is approximately 2.2 x 5 x 1.7 inches. This sampler can be used in about 75% of the river miles across the United States and also in many small lakes and reservoirs.

In large lakes or estuaries bottom sediment samples are usually collected with various types of dredges including the Ekman, Petersen and Shipek dredges. The Ekman dredge is widely and successfully used for soft bottoms. The Petersen dredge can be used for taking samples on hard bottoms such as sand, gravel, marl and similar materials. The Shipek dredge is a spring loaded sampler for use on all types of bottoms, but is especially useful in collecting and retaining the "fines" obtained from deposits of sand, silt or clay.

Numerous investigators prefer to collect core samples when interest is primarily in obtaining historical data, and provided the bed is sufficiently compacted to be sampled with a coring device. Though value of this type of sample should not be minimized, fresh deposition is of prime importance in a continuous evaluation of the occurrence, distribution and movement of pesticide residues.

Most core samplers lack positive seals to hold a core or moist sample in place as the sampler is withdrawn from the water. The Controlled-Depth Volumetric Bottom Sampler described by Jackson and the Core Sampler For In Situ Freezing of Benthic Sediments designed by Gleason and Ohlmacher are examples of efforts to overcome the drawbacks of the common core sampler and to provide the investigator with reliable equipment (1).

Little progress has been made in developing equipment to collect representative samples at the liquid-solid interface because it is difficult to sample without disturbance. The Gleason core sampler offers an advantage in sampling this interface because it can be used as a point sampler. The Hydrosol Sampler designed by Lawrence may be used in many sampling situations.

SAMPLE HANDLING AND PRESERVATION

Deteriorated samples negate all the efforts and cost expended in obtaining good samples. Cleansed, narrow mouth glass bottles equipped with Teflon-lined screw caps should be used for the water samples collected by the various depth-integrating samplers. Bottom materials containing a high percentage of water and composite water samples can be placed in cleansed, wide mouth glass bottles equipped with Teflon-lined caps. Plastic bottles must not be used since they are known to introduce interference and sorb pesticides. Alternately, solid bed-material samples may be sealed in foil. Sufficient sample should be collected to satisfy the volume requirements of each analysis and also to permit running of duplicate and spiked analyses. Breakage of glass sample bottles is overcome by shipping them in expanded polystyrene containers molded to fit the bottles.

The fieldman should provide the following information in writing for each sample: date, time, location, depth, type of sample, name of sample collector, and any other information that may be helpful in selecting the analytical approach or in interpreting the results. All samples should be kept near freezing, and extractions should be carried out in minimum elapsed time, preferably within 2 days from time of sample collection.

SAMPLING FREQUENCY

In conducting reconnaissance studies, defined here as short-term one-time evaluations, both bottom deposits and the overlying water should be sampled at each site. Monitoring, which consists of repetitive, continuing measurements to define variations and trends at a given location, should include collection of water samples during each of the four seasons, with particular emphasis on the fall and spring collections. Bed-material samples should be collected for analysis of fresh deposits at least once per year at monitoring sites, and preferably during both the spring and fall seasons.

Evaluation of the variability in available pesticide data must precede any decision as to the number of samples and collection frequency required to maintain an effective monitoring program.

REFERENCES

The Hydrologic Environment

1. Culbertson, J. K., and Feltz, H. R., "Sampling Procedures and Problems in Determining Pesticide Residues in the Hydrologic Environment," Pesticides Monitoring Journal, Vol. 6, No. 3, pp. 171-178 (1972).

2. Kittrell, F. W., A Practical Guide to Water Quality Studies of Streams, U.S. Federal Water Pollution Control Administration, 135 pp. (1969).

3. Guy, H. P., and Norman, V. W., Field Methods for Measurement of Fluvial Sediment, U.S. Geological Survey Tech. Water Resour. Invest. Ser. Book 3, Chap. C2, Washington, D.C. (1970).

4. Feltz, H. R., Sayers, W. T., and Nicholson, H. P., "National Monitoring Program for the Assessment of Pesticide Residues in Water," Pesticides Monitoring Journal, Vol. 5, No. 1, pp. 54-62 (1971).

5. Methods for Organic Pesticides in Water and Wastewater, U.S. Environmental Protection Agency, National Environmental Research Center, Cincinnati, Ohio (1971)

6. Goerlitz, D. F., and Brown, E., Methods for Analysis of Organic Substances in Water, U.S. Geological Survey Tech. Water-Resour. Invest. Ser. Book 5, Chap. A3, Washington, D.C. (1972).

CHAPTER V - ESTUARIES

The decision to monitor an estuary for pesticides may derive from any one or several specific needs. These needs or objectives will largely determine the character and *modus operandi* of the program. Obviously, two pesticide monitoring programs in the same estuary might be entirely different because of the kinds of information sought. Estuarine monitoring objectives may be for the purpose of determining:

1. Background levels of an array of persistent waterborne pesticides by randomized sampling of estuaries in a particular geographical area.

2. The escapement of pesticides in surface run-off from specific use areas in the drainage basin by sampling deltaic sediments.

3. The cause of increased faunal mortalities or lack of species diversity in an otherwise normal appearing estuary.

4. Tissue residue levels of persistent pesticides to ensure that they are within legal tolerance levels for edible fish and shell fish or their products.

5. Pesticide residues in food chain organisms to alert resource management agencies of possible mortalities resulting from trophic magnification.

6. Pesticide residues in pre-spawning gonads of commercially valuable species to identify causes of change in productivity.

The choice of which physical or biological elements are to be monitored in an estuary will be determined by specific program objectives.

Water samples taken at infrequent intervals or at limited points in an estuarine system will usually be of limited value. If the monitoring program objective requires specific knowledge of pesticide residues in the water, the guidelines enumerated in Chapter IV should be followed for such samples as well as for sediment samples.

Sediment samples are useful in detecting persistent pesticides in the estuary. However, interpretation of their analyses requires knowledge of particle size, organic/inorganic composition, station location with reference to current flow and similar data that frequently are not readily available. Sediments in shallow estuaries can be

disturbed by storm conditions and their pollution burden may change drastically in time of flood or drought without reference to pesticide usage in the area. Analysis of stratified estuarine sediments may reveal unusual patterns of pesticide residue accumulation, but our lack of information on aerobic and anaerobic degradation of persistent pesticides complicates the interpretation of such sediment samples. Carefully collected samples at the sediment-water interface along the geographic axis of an estuary may be useful, however, in pinpointing up-stream sources of pesticide pollution.

The choice of a biological sample for monitoring the estuary is determined largely by two factors: is the form to be sampled migratory, and what is its position in the trophic web? Sessile or non-migratory species, representing entire communities present obvious advantages since they reflect pollution levels at specific locations. Their life style usually facilitates age determination and permits some degree of bracketing of the occurrence of the pesticide pollution.

Many non-mobile species, e.g., molluscs and barnacles, are detritus and filter-feeders. Pesticide residues may be biologically retained and magnified in their tissues and reflect the introduction of pollutants into the lowest levels of the food web. Such residues suggest direct contact with pollution sources. Residues in vertebrate carnivores, on the other hand, are more likely to reflect trophic magnification of persistent pesticides. Fish-eating birds or mammals not only concentrate but store for long periods compounds that contaminate their diets.

Plankton offers several advantages as a tool for estimating levels of pesticide pollution. Its small particle size presents a relatively large sorptive surface. The cells are usually high in lipid content and readily take up organochlorine compounds. However, the rapid cell division and growth rates favor dilution of tissue residues. Also the interpretation of data on pesticide residues in plankton may be confused by the facts that plankton is a composite of living and dead materials that contains varying amounts of silt and other inorganic materials, and its moisture content varies widely depending on the components. Plankton samples containing pesticide residues are probably indicative of fairly recent contamination. When samples are taken at frequent intervals, they may be especially useful in identifying pollution sources.

Crustacea, such as commercial shrimp, are generally one of the least satisfactory groups to monitor because of their extreme sensitivity to insecticides. Both organophosphorus and organochlorine insecticides

will kill crustaceans at concentrations in the parts-per-trillion range. Carbamates are somewhat less toxic to them and herbicides generally are not toxic at concentrations likely to be encountered. The net result is that pesticide effects on crustaceans are likely to be an all-or-none affair and crustacean samples may reveal little about relative levels of pesticide pollution in the estuarine environment.

Molluscs (oysters, clams, and mussels) have special merit as bioassay tools because of their sensitivity to synthetic organochlorine pollutants present in the ambient water. They detect and accumulate these persistent pesticides to an astounding extent without being themselves markedly affected by the pollution levels generally encountered in estuaries. Not all molluscs are equally sensitive and, as with other bioassay animals, care must be exercised in comparing pesticide residue levels between individuals and species. Salt-water mussels are especially useful as monitor species because of their wide geographic distribution and their ubiquity over a broad range of salinity regimes.

The chief objection to the use of molluscs lies in their rapid metabolism of pesticide residues. Although they can concentrate pollutants in their tissues by a factor of 50,000 or more, these residues are lost in a matter of days when the ambient water becomes free of contamination. Thus, oysters and mussels are monitor animals of choice when sampling can be done on a monthly or more frequent basis. They are relatively useless in reflecting trends in environment pollution when sampling can be done only once a year.

Fish are often the most convenient group to monitor because of their availability from the commercial catch. They are sometimes sensitive to high pesticide residues in their environment as evidenced by massive fish-kills, but may accumulate relatively large residues and become pesticide-resistent when the concentration of a pollutant is low. Experimental work has shown that they accumulate pesticides directly from the ambient water as well as from their food supply. The interpretation of pesticide residue data in fish is difficult unless their life history is known.

Fish are highly selective in their diet and may accumulate small or large pesticide residues in polluted estuaries, depending on their food supply. Plankton-feeding or herbivorous fish tend to have significantly lower pesticide residues, for example, than carnivorous species that feed on small fish in the same estuary. Populations of even the same species of fish may have quite different diets in two different estuaries. Comparison of pesticide residues in these two populations could be very misleading if their food habits are not known.

Fish store organochlorine residues primarily in tissues having a high lipid content. Such residues accumulate as the fish age but may diminish sharply at spawning or in starvation periods when stored body fats are mobilized. Wide variations in pesticide residues may occur also in fish having presumably similar backgrounds. In one series of analyses of 15 "similar" specimens collected simultaneously from a school of estuarine fish, for example, DDT residues varied by two orders of magnitude.

Fish are most useful as monitors when they are small enough to permit whole body analysis of at least 15 specimens, thus averaging individual variations. If the fish are about 1 year in age or sampled prior to their first spawning, the residues reflect pollution exposure during a known time period.

Sample Preservation - The handling of estuarine samples for pesticide monitoring poses the same types of problems as samples from any other medium. Increased knowledge of the dangers of sample contamination through contact with various kinds of synthetic wraps and containers has demonstrated the necessity for glass and perhaps aluminum foil containers to preserve the integrity of wet samples. Immediate freezing and maintenance of frozen sample until analysis is still the best way to protect samples and prevent degradation or loss of pesticide residues. Freezing, because of its simplicity, is also perhaps the best method for use by unskilled personnel.

The preservation of tissue samples for pesticide analysis at room temperature by the use of desiccants has been used with marked success in estuarine monitoring. Although this method requires a modest amount of personnel training as well as special chemicals, it avoids the loss of frozen samples because of missed airmail schedules and power failure. It has proven especially useful for samples collected long distances from the analytical laboratory. In practice, field samples of plants or animals are chilled, homogenized, and blended with a combination of two desiccants - sodium sulfate and powered silica. The resulting mixture is a dry, free-flowing powder in which pesticide residues remain intact for 15 days or more at room temperature. This desiccant mixture is compatible with chromatographic procedures for organochlorine, phosphorus, and phenoxy pesticide compounds.

Representative Samples - The collection of representative field samples requries careful planning and pre-supposes a good understanding of estuarine ecology. The introduction of stratification into the selection of sample type, collection frequency, and station location is advisable if the most knowledge is to be gained from samples that are necessarily limited in number and areal coverage.

It has been found in past monitoring programs, that apparent pesticide pollution levels have been grossly altered or misinterpreted because of drought, windstorms, substitution of one species of clam for another, collection of samples on different tidal stages, and by assuming that the diet of a particular species of fish being monitored was the same in two different estuaries. Ultimately, the understanding of field sample analyses is determined by knowledge of the response of similar samples, either organic or inorganic, to pesticide pollution under controlled conditions. Too often, money and effort in environmental monitoring have been largely wasted because of the failure to select samples whose analyses could be meaningfully related to environmental conditions.

CHAPTER VI - FRESH WATER FISH

Monitoring of fish for pesticide residues is carried out because of their wide range of sensitivity to this group of pollutants, often demonstrating residue levels which are many fold greater than those found in water. Another reason for monitoring fish is that they are frequently used for human consumption and must therefore be free of unsafe levels of these contaminants. Except for a few species, however, commercial exploitation of fresh water fish for the market place would not justify the regulatory monitoring of many other species available. Since freshwater fish play a significant role in peoples recreational pursuit and since many other species of desirable wildlife rely on fish as their main food source, a knowledge of the level of pesticide contamination and its possible effects on fish populations is a necessary resource management tool. Thus, because of their widespread distribution, their opportunity for exposure to pesticides, their role in the national economy, and their importance in the web of life, fish are a logical organism for monitoring pesticide residues in the aquatic ecosystem. After determining the major objective for including fish in a monitoring scheme, specific decisions must be made as to how these objectives will be met. The following considerations are presented as a guide in making these decisions. Many of these considerations will be interdependent.

Complete standardization of representative fish species to be used in a monitoring program is difficult. No one species can represent all fishes. The availability of a given species for successive years, geographic location and background history are factors which dictate the selections. Species near the top of the food chains such as largemouth bass, yellow perch, channel catfish, green sunfish, rainbow trout and even carp are some logical choices because of their food habits or availability. Alternate species from the same fish family should be substituted, if necessary.

Where the mode of pesticide action is rapid, fish species should be selected near the top of the food chain so as to reflect maximum residues present. When long-term or chronic subtle effects of pesticides are to be studied, other species of fish and aquatic organisms representing various trophic levels may be included. Other considerations for selecting the species to be monitored have been discussed in the preceeding chapter.

The factors of fish size, age and numbers are considerations to be made. Uniform size and age of a single fish species should produce less varability in residue values. Erroneous residue readings will probably result in analyzing a composite of large, small, young and old, and intermixed species. Extremely large or old fish

should be avoided or should be analyzed as individuals. Fish which are in their first year or have not yet spawned represent the ideal age group for detecting recent contamination and are particularly suited for programs with sampling frequencies of one or more years.

Number of fish to be collected is somewhat arbitrary. Residue variations in the individual fish in a particular situation must be determined and will serve to establish the number of fish needed to constitute a statistically valid test. Availability of fish and costs of analysis will determine the numbers that are used. The National Pesticide Monitoring Program was established using 3-5 uniform sized fish in a replicated composite. Each composite is treated as a single sample. This is considered a minimal number of fish per sample. Ten to twenty individuals would be considered more ideal. Initially, depending on time or available funds, a cursory screening program may be adequate to gather information until refinements can be made.

Fish collections should be made using only electrical or mechanical means such as: seines, gill nets, trap nets, hook and line, electro-shocking equipment, etc. No fish-collecting chemicals are recommended. Fish collections must be made in conformance with state or local regulations and may require special or specific permits from the proper authorities.

A choice must be made whether to analyze whole fish or only the edible portions. Analyzing the whole fish is preferred where biomagnification aspects are concerned, such as in predator-prey relationships. Analysis of edible portions of fish is recommended for determining whether fish intended for human consumption contain unsafe levels of pesticide residues. If specific tissues are to be analysed then each fish must be handled separately, thus increasing time and costs of the program.

All samples should be chilled or frozen immediately for shipping to the laboratory. If whole fish are to be used for analysis then the field preparation of the sample will be minimal. Fish should be wrapped, either individually or as a composite sample, in clean aluminum foil and frozen as quickly as possible. If specific tissues or organ systems (brains, gonads, etc.) are to be analysed separately, they should be removed before such freezing, isolated in vials or other suitable container, labeled and rapidly frozen along with the rest of the sample. Such tissues cannot be removed after thawing without significant deterioration. If whole fish are to be used, all the fish in the sample should be ground up and homogenated and subsamples should be taken as needed. All samples should be protected from contamination after the samples are taken.

CHAPTER VII - WILDLIFE

The environmental circumstances under which monitoring of pesticides in wildlife may be warranted are sufficiently varied to preclude more than guidelines for developing monitoring plans and procedures. Further, with less than a decade of experience in wildlife monitoring, much is yet to be learned about the dynamics of pesticides in a diversity of wildlife populations. Thus the following guidelines are based on intuition as well as experience.

The term "wildlife", as used here, refers primarily to birds or bird parts and occasionally their eggs, but it may logically be extended to mammals and other terrestrial vertebrates. Certain fish or invertebrate species important in avian diets might be monitored as part of a total wildlife program; however, procedures for monitoring such forms are presented elsewhere in the report.

Although the "working" objective in virtually all wildlife monitoring is to assess body levels and trends of pesticides and related chemicals in given wildlife populations, both in time and space, specific objectives will vary among situations. Any optimal monitoring plan, including selection of the species to be monitored, will depend upon the predetermined need for monitoring and the specific objectives to be met. Clearly, an optimal plan cannot be formulated without first a precise statement of objectives and priorities.

A concept helpful in planning is to consider wildlife monitoring as being either species-oriented, region- or ecosystem-oriented, or perhaps a combination of such approaches. At times interest in the welfare of a particular species may justify its monitoring, in which case the survey design should be oriented toward sampling that population rather than specific areas or ecosystems. If the species of interest is too valuable to be sampled or for some reason is not readily available, a closely-associated "indicator" species might be substituted provided one can be found whose residue levels bear a strong relationship to the original species.

When regions or specific ecosystems rather than species are of primary importance, the species should be selected as an effective indicator of the relative degree of contamination of wildlife associated with the given environment. The species itself may or may not be of particular importance and, in fact, may advantageously be an expendable nuisance species (5,6).

SPECIES SELECTION

Selection of a "best" species for monitoring is seldom a simple matter, and at least the following factors, some more obvious than others, should

be considered in attempting to meet monitoring objectives:

Availability -- A species must be available in numbers sufficient to provide statistically adequate sample sizes. When animals must be sacrificed in monitoring -- the usual case -- availability infers: sufficient abundance to insure no harm to the population; expendability in that a highly desirable species is not used in lieu of a less valuable but fully suitable substitute; the species is sufficiently easy to capture to be practical.

If sacrifice is not prerequisite, as would be the case if blood samples could be used for residue analysis, then one need be concerned principally with ease of capture. The subject of sample size is discussed in detail in the chapter on statistics and study design. We stress at this time only that there is no fixed or quick answer to what constitutes an adequate sample. One must first specify the magnitude of the smallest difference relative to the mean he wishes to detect; the probability that he will detect such a difference if it exists (power); and the probability that his sample will erroneously indicate a difference when, in fact, none exists (type I error). He must also, from previous sampling, have an adequate estimate of the variance of the residue data to be encountered and the expected form of its statistical distribution.

Distribution -- If the primary interest is in monitoring residue trends on a regional basis rather than in a particular species, it is highly preferable that a candidate species occur throughout that region. Assuming only one species is to be used, any part of the region permanently devoid of that species cannot be considered as being included in the survey. When an otherwise suitable species is not available throughout an entire region, it may be possible to substitute an alternate species in those areas devoid of the principal species provided one can be found whose tendencies to accumulate residues are relatively similar. This procedure should be undertaken cautiously, however, and in no event should the two species be considered equivalent and their residue data indiscriminately combined.

Mobility -- The mobility of a species or, more precisely, the size of its home range, is a necessary consideration in determining if the species will serve to meet the objectives of a survey. One must consider the extent of daily and seasonal movements, and if a migratory species, the timing and pattern of migration as well as the extent of movement at either end of the migration route. If the monitoring objective is to estimate residue tendencies over a rather broad area, one may utilize a more mobile species than if he wishes to associate residues with specific areas. Use of migratory species to associate residues with particular areas obviously poses special problems, and if a suitable resident species is available, the latter will in all likelihood be the better choice. Any attempt to use a migratory species should be undertaken cautiously and with adequate knowledge of migration patterns and rates of residue accumulation in that species.

Diet -- Food habits are a primary consideration in selection of a species, since the principal route of pesticidal exposure in the wild is undoubtedly through the diet. Because persistent pesticides tend to become progressively concentrated through successive levels of food chains, a species' food habits must be representative of the segment of the environment at which the monitoring is primarily directed. Because of food chain concentration, or "biological magnification", herbivorous species in a given ecosystem will tend to be exposed to lower levels of pesticides than will omnivorous species, and they, in turn, will be exposed to lower levels than carnivorous ones. In particular, fish-eating animals will be exposed to the highest residues because of eventual translocation and buildup of persistent residues in aquatic systems. With reference to the factor of mobility, there is a decided tendency for carnivorous species to range more widely in search of food than do herbivorous species. Thus, if the investigator wishes to monitor a species with a restricted home range, his choice may be limited to herbivorous animals with restricted exposure to environmental residues. In such instances, some compromise between food habits and size of home range may be necessary.

Habitat -- The habitat preference of a species is an obvious factor to be considered and little elaboration here seems necessary. Clearly, if the intent of monitoring is to evaluate residues in a wetland ecosystem, the investigator does not select an upland species. In practice, however, the habitat factor may involve something more than a simple dichotomy and require greater scrutiny.

Physiology -- The physiology of a species must be considered with regard to its propensity to acquire and retain residues of those pesticides to be monitored. Such information may be available in the literature, otherwise, experimentation prior to operational monitoring is strongly recommended. A species uniquely able to rid itself of given residues may not be satisfactory for monitoring.

THE MONITORING PLAN

Following the selection of species, a detailed monitoring plan must be developed that conforms with limitations of budget, personnel, and operation logistics. The plan must be based on statistically sound sampling procedures if data interpretation is to be meaningful. It must also include such details as frequency of sampling, required sample size, method(s) of capture or collection to be used, and instructions for handling specimens from time of collection through chemical analysis. Careful consideration should be given to the type(s) of statistical analysis to be employed so that there is compatability with the sampling protocol.

Randomization -- To obtain, from a given animal population, a sample that is known to be absolutely random is perhaps an "impossible dream". Nonetheless, the investigator must strive, to the best of his ability, to achieve randomness in his sampling. Certainly he should keep his sampling methods consistent from period to period, so that any bias due to sampling should remain relatively constant among periods and thus have little effect on measurements of trends in residue levels.

Sampling Approaches -- Basically, there are two approaches to obtaining specimens for monitoring: Specimens may be collected in the field specifically for monitoring; or they may be sampled from some collection unrelated to monitoring, such as the hunting bag. When sampling from the field, it is the sampling site that must be chosen at random as it is virtually impossible to select the individual animal at random. It should be emphasized that even though more than one specimen be taken from a site, it is the site, not the specimen, that is the primary sampling unit; and degrees of freedom for estimating survey precision are based on total sites, not total specimens. When sampling from a collection of specimens unrelated to monitoring, one can sample only the collection at random and thus obtain unbiased estimates of residues in the collection. Since the collection itself is not likely to be random, it must be assumed that future collections will be made in a similar manner and that any biases will be relatively constant among collections.

When an objective for monitoring is to estimate residue levels and trends for specific subdivisions of populations, stratified random sampling is called for. The subject of stratified sampling is too extensive to be more than mentioned in this section, except to point out that strata must be established before actual sampling has occurred; one may not construct strata after the fact.

Sex and Age Differences -- An important consideration in wildlife monitoring design is whether a species' capacity to accumulate certain residues differs according to sex and/or age of the animal. For example, fall levels of DDE in wings of mallards and black ducks have been shown to be higher for adult than for immature birds (1). There is evidence that wings of adult male mallards and black ducks may develop higher residues of DDE than adult females (4), possibly because of partial purging of the chemical via the egg during laying. In experiments with bobwhite, mirex was shown to be readily eliminated via the egg to an extent that after eight weeks of laying, carcass residues in male breeders were about twelve times as high as those in the females (3). If sex or age differences in residue levels are likely to be meaningful, either the particular groups should be sampled separately, or the sample size should be sufficiently large to insure the likelihood that all groups are properly represented.

Frequency of Sampling -- The frequency with which monitoring should be repeated to meet objectives most efficiently is best determined uniquely for each program. Monitoring is not an inexpensive operation, and frequency should obviously be kept to the essential minimum. At times the expectation of seasonal differences in residue levels may be of sufficient importance to justify monitoring more frequently than once a year. Conversely, it is conceivable that trends would be sufficiently gradual to require monitoring only once every two or three years. Perhaps the soundest approach is to monitor, at the onset, as frequently as might be necessary and fiscally possible, and then reduce the schedule to the frequency found to be sufficient.

Methods of Capture -- Wild specimens may be collected in the field using a variety of techniques, which may be categorized basically as shooting, live- or kill-trapping, netting, or capture with drugs. A detailed account of such techniques and further references may be found in "Wildlife Management Techniques, Third Edition" (7). Wildlife collections must be made in conformance with all applicable laws and regulations and may require special or specific permits from the proper federal, state or local authorities.

To be satisfactory, a capture technique must be practical, it must not contaminate the specimen with any substance that might interfere with pesticide analysis, and it must not introduce sampling bias in being more selective of one segment of a population than another. If, for example, one sex or age group is more readily captured than another and groups are not stratified, or if there is a correlation between body residue burden and ease of capture, then a biased sample is the inevitable result. There is seldom a ready means of determining whether a particular capture technique will be biased under given conditions, and perhaps the best one can do is to combine experience and the literature with the best logic he can muster to minimize the likelihood of this bias.

Constructing the Tissue Sample -- The manipulation of specimens following collection constitutes an important phase of the monitoring plan. For example, is it advantageous to quantify residues in the whole body, in the skinned, eviscerated carcass, or in some particular organ or tissue? Is it most efficient to analyze specimens individually, to "pool" the specified tissues of several animals and analyze the homogenized tissue-pool, or is it best to use a combination of both approaches? There are no ready answers to these questions: each will depend on the species being sampled, specific monitoring objectives, and perhaps budgetary limitations.

In estimating environmental trends, a reasonable approach is to analyze the whole body, less visceral contents, and thus quantify the total body burden. A further refinement might be to remove feathers or hair and skeletal protuberance such as the beak and feet of birds. An alternative to the tedious job of plucking birds is to skin them; however, skin is high in lipid material which may contain significant amounts of fat-soluble pesticides. For monitoring some chemicals, it may be advantageous to analyze specific

organs rather than entire carcasses if such organs are known to be foci of residue accumulation.

When the only realistic way to monitor a species is to use body parts, such as wings or legs of animals bagged by hunters, it is essential that residue levels in such parts be highly correlated with residues in the total body. The literature may contain such information for a few species; but most often, tests must be conducted prior to monitoring to determine whether or not a satisfactory relationship exists.

Pooling Specimens for Analysis -- When the primary objective in monitoring is to measure trends in mean residue levels, as is generally the case, a given level of precision may be obtained at a reduced cost by pooling specimens for chemical analysis. If, for example, an investigator collects 10 specimens from a given site, he might analyse each specimen individually (10 analyses) or he might first chop and blend the 10 specimens into a single homogenate and make a single analysis of the homogenate. In both instances he is estimating a mean residue level in a sample comprising 10 specimens, but he does so at about one-tenth the cost by pooling. Assuming random sampling from a given population, the variance of mean residue levels in pools of ten specimens should be one-tenth the variance of ten individual specimens.

The disadvantage to pooling is that little if anything can be learned about the frequency distribution of residue levels in the population. Pooling, at best, offers only partial answers to questions such as: Is the distribution skewed to the right, as is frequently the case, and, if so, how badly? What are the extreme levels in the sample, and are they sufficiently high to be detrimental? A reasonable compromise is to analyze a sufficient number of specimens individually to examine residue distributions and a sufficient number of pools to estimate mean residue levels with adequate precision.

THE NEED FOR TRIAL MONITORING

Because of the number of variables and probable unknowns to be encountered in establishing a monitoring program, there is much to be said for testing proposed procedures with a trial program (2). The scale of the trial need only be large enough for an adequate test of procedures and will probably depend more on the extent of uncertainties than on the size of the proposed program. In the extreme, trial monitoring may show the proposed program to be untenable, with resultant savings of significant magnitude. Most often the trial will permit adjustments of procedures and sample size determinations that should justify the expense of the trial.

WILDLIFE REFERENCES

1. Heath, R. G. 1969. Nationwide residues of organochlorine pesticides in wings of mallards and black ducks. Pestic. Monit. J. 3(2):115-123.

2. Heath, R. G. and R. M. Prouty. 1967. Trial monitoring of pesticides in wings of mallards and black ducks. Bull. Environ. Contamination Toxicol. 2(2):101-110.

3. Heath, R. G. and J. W. Spann. 1973. Reproduction and related residues in birds fed mirex. pp. 421-435. In Pesticides and the Environment: A Continuing Controversy, Vol. II. Edited by Wm. B. Deichmann. Inter-continental Medical Book Corp., N.Y., N.Y. xiii + 578 p.

4. Heath, R. G. and S. A. Hill. 1974. Nationwide organochlorine and mercury residues in wings of mallards and black ducks during 1969-70 hunting season. Pestic. Monit. J. 7(3/4):153-164.

5. Martin, W. E. 1969. Organochlorine residues in starlings. Pestic. Monit. J. 3(2):102-114

6. Martin, W. E. and P. R. Nickerson. 1972. Organochlorine residues in starlings - 1970. Pestic. Monit. J. 6(1):33-40.

7. Wildlife Management Techniques, Third Ed. 1969. Edited by R. H. Giles. Edward Bros. Inc., Ann Arbor, Mich. vii + 623 p.

CHAPTER VIII - FOODS AND FEEDS

This chapter is intended to provide a brief overview of the principals of sampling, the form of resulting data and the statistical aspects of data analysis relative to pesticide residues in foods and feeds. General observations are also made relative to computer support and to field sampling procedures.

The general types of food and feed sampling include:

Regulatory (or selective) sampling to prevent the introduction of over-tolerance products, both domestic and imported, into the food supply.

Surveillance (or objective) sampling to identify suspect shipments and emerging problems which should be subjected to regulatory sampling, and to derive a data base on the occurence of pesticides in individual food and feed commodities.

Trend sampling (FDA total diet or market basket studies) to identify shifts or other trends in food pesticide levels relative to existing dietary intake patterns. These data are derived from food prepared for the dinner table.

Products subject to pesticide sampling range from raw agricultural commodities to processed and packaged foods and feeds. Two general types of sampling procedures are normally involved, those for bulk sampling and those for unit package sampling.

GENERAL SAMPLING PRINCIPLES

There are several basic considerations in sampling that apply to the problem of pesticides in food and feeds.

Statement of Objectives

A precise statement of the objectives desired from the sampling program must be derived. For example: the objective might entail coming to a well founded decision relative to regulatory action or in another case merely to identify gross trends in pesticide levels. Sampling results in one situation may not be adequate for the objectives of a different situation.

Definition of the Population

The term, population, refers to the aggregate of units about which a decision is to be made or an inference in drawn. In some cases the population will be readily identifiable physical collection of units (e.g., a production lot in a warehouse consisting of a specific

number of units such as bags, cans, or other units). In other cases the units may have to be defined in the problem context (e.g., the number of grams of food product consumed daily during a one week period by an individual). Sometimes the target population cannot readily be sampled and a somewhat different actual population must be used (e.g., units of food product consumed might have to be approximated by units of food products purchased). When the target population cannot be sampled directly it is important to recognize this fact and adjust inferences from the sample data accordingly.

Required Data Definition

Careful definition of the required data can result in sampling economies as well as in attaining program objectives. If information is desired only relative to the occurrence of units with pesticide levels in excess of tolerance levels less effort is required than would be if more detailed data are required.

Derivation of Required Sampling Precision

Inferences about pesticides in the population to be sampled depend on the type of sample, sample size and pesticide level variability. Therefore the precision required for the inferences sought are dictated by these sampling factors. In estimating a population parameter the precision required can be stated in terms of an acceptable degree of error and desired level of confidence (e.g., estimating the average pesticide level in a production lot within 1% of its true value with 95% confidence). For hypothesis testing situations (e.g., is regulatory action indicated) precision can be defined in terms of the producer's risk (Type I error) and consumer's risk (Type II error). Acceptable levels of these risks must be established during the planning stage.

Definition of Measurement Methods

Sample data are useful only within the limits of the measurement methods employed. In some cases, the most accurate pesticide analytical methodology may be required for individual population units whereas in others a gross screening method on composited units may suffice. The pesticide analytical methodology alternatives should be carefully assessed relative to precision, accuracy, time and cost and decision made consistent with program objective.

Construction of the Frame

As indicated above, the population definition is dependent in turn on the definition of the units making up the population. The frame is the identified list of sampling units. In production lots of packaged products this will be merely the individual containers, a small set of containers or perhaps a case of containers. However, in bulk sampling of foods (e.g., a car load of wheat) the sampling

units may well be dictated by the mechanical device used to collect the sample (e.g., the contents of a grain probe). Construction of the frame is essential to the application of random sampling procedures.

Sample Collection Requirements

With the preceding problem defined it is possible to specify to some degree the sample size and the type of sample required. Consideration of the time and costs for the sample collection may then dictate some modification of the problem objective.

Conduct of a Pretest

It is often of considerable use to conduct a preliminary small scale sampling exercise. Such a pretest will yield, for example, estimates of pesticide variability which will permit more accurate determination of sample size requirements. A pretest can often also identify problems in field interpretations, analytical methodology and other facets which can be resolved before the overall exercise is initiated.

Description of Field Procedures

Careful thought (and if possible a pretest) should be devoted to the field instructions relative to sampling site, physical collection of the sample, sample unit identification, preservation of the sample, packaging and shipping, etc. If pesticide analyses are to be made in field laboratories, detailed methodology instructions (method, equipment, sample handling, data recording, etc.) must be provided. Preferably specially designed data reporting forms should be used, which will facilitate data analysis and resolution of any questionable selection of the sample units.

Analysis of the Data

If the survey or sampling program is properly planned, procedures for the analysis of the data will have been detailed ahead of time. Analysis should first involve a review of the raw data for omissions, recording errors or other inconsistencies. Pesticide levels in foods and feeds will in general be highly variable depending on the food product involved, source, geography, degree of processing, etc. Distributional forms of pesticide residue levels in a given situation will usually be quite definitely skewed (i.e., most values very small with the frequency of occurrence decreasing rapidly as the values increase numerically). Analytical treatment of such findings can be made by using the lognormal distribution as the reference model. For the usual statistical tests of significance or estimation problems, observed pesticide data can be transformed by taking logarithms of the values and treating the transformed data in accord with normal (Gaussian) distribution theory.

The types of statistical techniques that will find use in pesticide problems include acceptance sampling (e.g., for regulatory decisions relative to domestic and import sampling) or analysis of variance (for identifying differences in pesticide levels as a function of various factors such as source, type or level of processing, brands, style, etc.). Trend analysis and related correlation/regression techniques will apply in those situations where relationship of time or other factors to pesticide levles are of interest.

A most important consideration in the analysis of food/feed pesticide data is the variability in the sample data. Variability arises from two major sources (1) sampling error and (2) method error. Sampling error arises from the fact that we have access only to data from a portion of the population and of course different samples from the same population will yield differing numerical values. Method error refers to the less than perfect capability of the analytical procedure to provide the true pesticide level. Any analytical method should then have associated with it statistical measures of its accuracy and precision. Estimates of variation associated with the method as supplied by different laboratories and different technicians have been derived through collaborative studies for several analytical methods.

Sampling errors will automatically be included in the statistical procedure (e.g., identification of the probability of getting a violative sample from a non-violate lot in acceptance sampling). However, special provision must be made for analytical error in many cases (e.g., a violative pesticide value may in fact be non-violative when method error is taken into account) such as in regulatory activity. In other situations it will be less important (e.g., a relative comparison of pesticide levels between two populations).

Computer Support

In most surveys or surveillance problems of food pesticide levels, sufficient data will be generated so that analysis is facilitated by computer treatment. It is also considered of value to retain data from past surveys. Access to such data is facilitated by computer treatment. Normally agencies concerned with pesticide problems associated with food will have access to medium to largescale computers. Therefore, equipment exists for convenient treatment but the program for storage and retrieval and for analysis will probably have to be developed or adapted in each case.

CHAPTER IX - HUMAN TISSUES

This discussion will be limited to the monitoring of humans for pesticides. It is recognized that many of the points discussed must be common to any program monitoring humans for other chemical, physical, or infectious agents. While human monitoring is similar in many respects to other environmental monitoring, it is quite different in a number of both technical and ethical aspects. Indeed, human monitoring may be the most difficult to perform meaningfully.

The following questions must be answered as the first step in planning a human monitoring program:

1. What use will be made of the collected data? By whom?

2. What population will be studied? How is it distributed geographically, by age, sex, race, health condition, occupation, cultural mores, etc.? What is the total potential population for study?

3. What other information is available concerning the group to be studied and how much of it will be coordinated with the monitoring data?

4. How often will samples be collected from the same individuals?

5. What chemicals including metabolites, are to be studied? Are analytical procedures available which are adequately sensitive and specific for the study requirements?

6. What laboratory support is available? What is its work capacity, technical competence, and quality control program?

7. What time interval can be tolerated between sample collection and data availability?

Clearly the answers to these questions will shape the final program plans.

Man is monitored in order to estimate his total exposure to one or more pesticide chemicals. This is a much simpler approach than is the direct measurement of exposure by all routes, and it is the only approach which permits survey of a sizable population group.

The problems of ambient monitoring are quite different from those of occupation-linked monitoring with regard to almost all of the preceeding questions. In general, occupational monitoring is easier to perform since the potential study group is usually limited by sex, age and health condition, and to some extent, geographically and socio-economically. Such a group is exposed to occupationally encountered

pesticides and may also be exposed through avocational activities to the same or different pesticides.

Ambient monitoring is generally concerned with minute traces and may produce negative results unless carefully planned as to chemicals to be studies, materials to be analyzed and sample collection procedures. Unlike sampling other environmental substrates, consideration must be given not only to the technology of the actual sample collection but also to the legality and ethics of sample collection as well as to the inherent limitation on the size of sample which it is feasible to obtain.

In order for ambient human sampling to reflect the condition of the population, the sample group must reflect the demographics of the population, i.e. sex, race, age and geographic distribution. Definition of the study population may restrict it in a number of specific parameters thereby increasing the standardization of the group. This definition must be done carefully in order to avoid biasing the entire study. Furthermore, in presenting and interpreting data from the study, the definition of the study population must be clearly reiterated in order to avoid unjustified and potentially erroneous extrapolation. Census data can be most helpful in identifying age, sex, race and geographic demographics by city, county, state, region or nation and should be reviewed before designing the study.

The necessity of monitoring a biologic creature further complicates the problem, requiring careful selection of the tissue or fluid to be sampled. Some pesticide chemicals and/or their metabolites are stored in the tissues of the body at different concentrations for long periods of time, others are rapidly metabolized and excreted a short time after exposure as unique products or as products indistinguishable from those of normal metabolic processes. Since the original level of exposure is usually quite low in the general population, the stored or excreted levels must also be low. Normal body components or other materials to which there has been exposure may also complicate analysis. Analytical technology does not permit the measurement of all pesticide chemicals and metabolites in a single sample of tissue or fluid, therefore, more than one tissue may be required from each individual.

Samples from the human body, whether tissues, blood or excreta, are not easily obtained. The question of informed consent must be resolved for each individual.

Sample identification and handling are other areas which require pre-planning decisions. Usually a small amount of information is desirable about the sample donor, but this should be kept to a minimum, being limited to those facts for which there is a demonstrated need. The donor's identity must remain anonymous when the data is

evaluated. Sample container, sampling technique and sample storage and transport are important in preventing the introduction of extraneous and interfering materials which further complicate the interpretation of analytical results and in preserving chemicals within the sample. Details of these procedures must be verified with the supporting laboratory before sample collection is initiated. At the same time the reporting terms and format should be finalized for each type sample collected in units, total sample or extracted weight basis, before or following adjustment of osmolality, hermatocrit or pH, etc. Confirmatory tests to be used should be preselected since this will be important in determining the size of each sample collected.

Within this context, the following guidelines are offered for ambient human monitoring within any geographic subdivision:

1. Identify the intended use of data from the study

2. Identify the geographic area of the study

3. Define the study population by size, geographic distribution, age, sex, race, occupation, ethnic customs, etc.

4. Develop statistical design based on study population parameters to assure the collection of representative samples on a random basis. This should provide confidence levels and define acceptable margins of variation.

5. Determine pesticide residues to be monitored and materials to be collected and analyzed, also amount of material required for "a sample."

6. Select identifying information to accompany each sample.

7. Define sample collection, handling, storage and transport procedures, including procedures for assuring use of uncontaminated sample containers.

8. Identify sampled tissues.

9. Identify sampling locations geographically and make appropriate contacts. Informed consent procedures must be worked out with each sample provider.

☆ U. S. GOVERNMENT PRINTING OFFICE: 1975—626-592/216 3-1